Author’s response to reviews

Title: Circ-SMARCA5 suppresses progression of multiple myeloma by targeting miR-767-5p

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Author’s response to reviews:

Dear Editor and Reviewers,

Thanks for your comments on our manuscript BCAN-D-19-00032, entitled “Circ-SMARCA5 suppresses progression of multiple myeloma by targeting miR-767-5p”. We have amended the relevant part in manuscript in accordance with your comments and highlighted them in red color. The comments were answered below item by item as well.

Editor Comments:

1. Please include the email addresses for all authors on the title page. The corresponding author should still be indicated.

   Answer: Thank you for your suggestion. The email addresses for all authors were added on the title page. The corresponding author was indicated.

2. Please move the Acknowledgments and Competing Interest information from the title page to the Declarations section.
Answer: Thank you for your suggestion. The Acknowledgments and Competing Interest have moved to the Declarations section. Thanks again for your suggestions.

3. Please have the text edited by a professional language editing service or a native English speaking colleague. There are many issues with grammar, wording, spelling, and/or punctuation that need to be addressed.

Answer: Thank you for your suggestion. We have invited an English language editing company American Journal Experts (https://www.aje.cn/) to check for the issues you have mentioned and polish this article.

4. Please add a “Conclusions” section after the “Discussion” section. This should state clearly the main conclusions of the research article and give a clear explanation of their importance and relevance.

Answer: Thank you for your suggestion. The “Conclusions” section was added after the “Discussion” section. Thanks again for your suggestions.

5. Please note that all manuscripts must contain all the following sections under the heading 'Declarations'. The Declarations should follow the Conclusions section, and be before the References.

Answer: Thank you for your suggestion. The Declarations was followed the Conclusions section, and be before the References. Thanks again for your suggestions.

6. Please provide a list of all the abbreviations used in the manuscript. This list should be placed just before the Declarations section. All abbreviations should still be defined in the text at first use.

Answer: Thanks for your comment. As you required, we have added a list of abbreviations before the Declaration Section.

Reviewer reports:

Nicola Amodio (Reviewer 1): The authors investigate the prognostic and biological significance of circ-SMARCA5 in MM.
The topic of the manuscript is interesting and add new insight to the pathobiology of multiple myeloma.

I have the following concerns:

- It is not clear the rationale of the study and why the authors focused on circ-SMARCA5.

Answer: Thanks for your comment. In order to make our rationale clearer, we have added more clues about Circ-SMARCA5 in cancers and added to the Introduction Section. The detailed description was “Circ-SMARCA5 is previously shown to participate in carcinogenesis by inhibiting cell proliferation, migration and invasion in several solid tumors including hepatocellular carcinoma and cervical cancer. Whereas in hematological malignancies, the role of Circ-SMARCA5 is unknown.”

- Please increase description on non-coding RNAs and circ-RNAs in the Introduction section.

Answer: Thanks for your comment. As you suggested, we have added more description on non-coding RNAs and circ-RNAs in the Introduction Section. The detailed description was “Non-coding RNAs, a category of RNA molecules that are not translated into proteins, are widely abundant in human genome and engage in numerous cellular processes including transcription, post-transcriptional modification and signal transduction. Circular RNAs (CircRNAs) comprise a class of non-coding RNAs whose 3’ and 5’ ends are joined together forming a covalently closed loop. Benefiting from the structure, circRNAs are resistant to exonuclease-mediated degradation and are more stable compared with linear RNAs.”

- The cutoff for high and low circ-SMARCA5 in myeloma patients should be explained.

Answer: Thanks for your comment. The cutoff value of Circ-SMARCA5 high and low expression was the median value of Circ-SMARCA5 in MM patients at baseline. And to avoid confusion, we have added the relative description to Result Section of manuscript under subtitle “Association between Circ-SMARCA5 relative expression and patients’ baseline characteristics”, as well as to the corresponding Figure legends.

- The nomenclature NC-, NC+, circ+ and circ- should be changed because it is not easily understandable for readers.

Answer: Thanks for your comment. As the nomenclature were not easily understandable, we have changed them from “NC-, NC+, circ+ and circ-” into “Control (-), Control (+), SMARCA5 (+) and SMARCA5 (-)” in the manuscript.
-it should be appropriate that gain and loss of function experiments are carried out in different cell lines, respectively with low and high circ-SMARCA5 levels.

Answer: Thanks for your comment. As you required, we have performed additional gain and loss of function experiments in high circ-SMARCA5 level cell line, NCI-H929 cells. And we observed similar results to the experiments performed in RPMI8226 cells. The relative description was added to the Result Section, and as Figure 7-revised. The order of all the figures was rearranged.

-Blots are not properly cut, and densitometric analysis should be reported.

Answer: Thanks for your comment. Regarding to your comment about the Western blot, we have revised the western blot results for a proper cut and added the protein grey scale quantification as Figure 6D, 6E and Figure 10D, 10E. The relative description was added to Method and Result Sections as well.

-The term "compensation experiments" is uncommon and should be replaced.

Answer: Thanks for your comment. As “compensation experiments” is uncommon, we have replaced it with “rescue experiments” in our manuscript.

Mu Hao (Reviewer 2): REVIEWER COMMENTS FOR THE AUTHOR:

Reviewer:

In this present study, authors demonstrate that Circ-SMARCA5 is correlated with better prognosis of MM, and it inhibits cell proliferation and promotes cell apoptosis via sponging miR-767-5p. The finding is interesting and need to explore more to provide us solid evidences of Circ-SMARCA5 function and mechanisms involved in pathogenesis of myeloma.

Major comments:

1. The data need to be well reorganized and make them more clear which will be easier for reader to follow. The language should be written in a simple and declarative way and texts need to be more concise.

Answer: Thanks for your comment. According to your suggestion, we have reorganized our data by bolding the statistically significant results, simplifying the annotation of Tables, zoom in the figures, changing the arrangement of figures and adding annotations to figures in figure legends. As for the language, we have proofread the whole manuscript for grammar mistakes, shortened
long and complex sentences and simplified the narration as you required. In addition, we have also invited a English language editing company American Journal Experts (https://www.aje.cn/) to polish this article.

2. Figure 3. The cutoff value of Circ-SMARCA5 is not clear in Figure 2 and Figure 3. And how many patients in high and low level group are not clear as well.

Answer: Thanks for your comment. The cutoff value of Circ-SMARCA5 in Figure 2 and Figure 3 was the median value of Circ-SMARCA5 in MM patients. And the number of patients was 53 (50.4%) in high expression group and 52 (49.6%) in low expression group. For better understanding, we have added the above information into the Figure legends.

3. It is not clear about the control cells used in this study. Such as Figure 4. Authors compare wild types with resistant cells of MM cell lines to compare the differential expression of Circ-SMARCA5, otherwise bortezomib sensitive and resistant cell lines.

Answer: Thanks for your comment. As you pointed out, the control cells used in this study was not clear. We would like to explain that the relative description about the control cells was stated in Method Section under subtitle of “Cells culture”. The detailed description was “normal plasma cells were isolated from bone marrow of healthy donors using CD138-coated magnetic beads (Miltenyi Biotec, Germany) as normal control.”

In addition, the MM cells used in this study were wild-type MM cell lines, which was not mentioned in our original manuscript. In order to clarify this, we have added the description about MM cell lines in Method Section under subtitle of “Measurement of Circ-SMARCA5 in MM cell lines and normal plasma cells” in the manuscript.

4. Figure 5. Which kind of cell was used in this experiment? How to explain there are significantly decreased expression of Circ-SMARCA5 in Circ (-) group compared with NC (-) group?

Answer: Thanks for your comment. The experiment in Figure 5 was conducted to assess the transfection of Circ-SMARCA5 overexpression and Circ-SMARCA5 shRNA plasmids. And this experiment was carried out in RPMI8226 cells, because it was the cell line that expressed the lowest level of Circ-SMARCA5 among the MM cell lines used in this study. However, according to reviewer Nicola Amodio’s suggestion, we have conducted additional gain and loss of function experiments in NCI-H929 cells (Figure 7-revised).
As for your question that how to explain there were significant decreased expression of Circ-SMARCA5 in Circ(-) group compared with NC (-) group, the reason was that: In untreated RPMI8226 cells, there was a certain level of Circ-SMARCA5 expression. And NC(-) group was transfected with blank shRNA, in which the level of Circ-SMARCA5 was unaffected. However, the Circ (-) group was transfected with Circ-SMARCA5 shRNA plasmids, which silenced the expression of Circ-SMARCA5 in RPMI8226 cells. Therefore, Circ-SMARCA5 expression was lower in Circ (-) group compared with NC (-) groups, and this also indicated the successful transfection of the plasmids.

5. Figure 6. A did not show the proliferation inhibition in overexpression Circ-SMARCA5, but B shows increased apoptosis of those cells. How to explain the un-consistent result?

Answer: Thanks for your comment. You mentioned that in Figure 6A, the proliferation inhibition in circ-SMARCA5 was not shown in Circ (+) group compared with NC (+) group, which was inconsistent with the result in Figure 6B. We are afraid that Circ (+) did presented reduced cell proliferation compared with NC (+). In Figure 6A, the squares represented Circ (+) group and round dots represented NC (+) group, and at 72 hours after transfection, position of the square was lower than the round dot. Circ-SMARCA5 overexpression inhibited the cell proliferation compared with NC (+), which was consistent with the result in Figure 6B.

The presentation of Figure 6A was confusing so that it would cause misunderstanding of results. Therefore, we have zoomed in the symbols in Figure 6A and made it clearer to read.

6. Figure 7. It is weird that the level of Mir-767-5p is significantly upregulated in Circ (-) group than NC (-) group. How to explain the results?

Answer: Thanks for your comment. In Figure 7C, miR-767-5p expression was reduced in Circ (+) group compared with NC (+) group, and increased in Circ (-) group compared with NC (-) group. This was be due to that Circ-SMARCA5 reversely regulated the expression of miR-767-5p by acting as microRNA sponge. When the presence of Circ-SMARCA5 was eliminated, the expression of miR-767-5p would increase. And this was further validated in our luciferase reporter assay, which showed the direct interaction between Circ-SMARCA5 and miR-767-5p.

7. All of the detection of Circ-SMARCA5 and Mir-767-5p are performed on the MM cell lines. Authors should detect the level of primary patient samples to validate this finding.
Answer: Thanks for your comment. As you pointed out, the detection of Circ-SMARCA5 and miR-767-5p are performed on the MM cell lines, and their levels should be detected in patient samples for validation. We have to explain that this study consisted of two major parts: clinical part based on patient samples and cellular experiments. And in study design of the clinical part, we did not take miR-767-5p into consideration because its interaction with Circ-SMARCA5 was unknown until the cellular experiments were done. Therefore, miR-767-5p was not detected in patient samples in this present study. However, our findings regarding the interaction between Circ-SMARCA5 and miR-767-5p in MM cell lines is a valuable reference for the future study, which is scheduled to detect Circ-SMARCA5 and miR-767-5p in patient samples of MM.

8. The authors show provide some clinical evidence whether the high level of Circ-SMARCA5 in MM cells is more sensitive to the clinical treatment?

Answer: Thanks for your comment. Regarding whether the high level of Circ-SMARCA5 in MM cells is more sensitive to the clinical treatment, there have been clinical evidence in Figure 2 that in Circ-SMARCA5 high expression group, the number of MM patients achieved CR is higher than patients with non-CR. In addition, in Table 5, univariate logistic regression analysis revealed that Circ-SMARCA5 expression was positively correlated with CR rate in MM patients. These are the clinical evidence for the correlation between Circ-SMARCA5 high expression and high sensitivity to clinical treatment. The relative description about this was presented in Discussion Section as “Additionally, we also investigated the prognostic value of Circ-SMARCA5 in MM patients and discovered that Circ-SMARCA5 high expression was correlated with better treatment response to chemotherapy and longer survival, indicating that patients with Circ-SMARCA5 high expression were more sensitive to clinical treatment.”

Minor comments:

A. Check spelling, words and grammar throughout document. For example,

Answer: Thanks for your comment. We have checked the spelling, words and grammar throughout the document and invited a language editing company American Journal Experts (https://www.aje.cn/) to polish this article.

B. It is strongly suggested that the title should not be a so long sentence with punctuations. It should be shortened and become much clear and concise.
Answer: Thanks for your suggestion. We have shortened the title to a clearer and more concise version: “Circ-SMARCA5 suppresses progression of multiple myeloma by targeting miR-767-5p.”

C. Do not to start a sentence with numbers or lowercases, for instance, Methods CCK8 10 ul, qPCR in measurement of Circ-SMARCA5 in MM cell …, Results 105 MM.

Answer: Thanks for your comment. We have amended the sentences that started with numbers or lowercases, and double checked for other parts in the manuscript.

D. Under Results, I suggest that authors put the Table 1 and 2 to the supplemental data, or you can just transfer them to words and put them in the methods. Because those are not the results for the paper and not necessary to be isolated tables.

Answer: Thanks for your suggestion. Since the original Table 1 and Table 2 are not the results of the paper, we have changed them to Supplementary Table 1 and Supplementary Table 2.

E. In Table 4, Immunoglobulin subtypes instead of subtype; cytogenetics abnormalities instead of cytogenetics abnormality. Try to double check the proper use of plural nouns.

Answer: Thanks for your suggestion. We have amended the use of plural nouns as you pointed out and checked other parts in the manuscript.

F. In Table 5, there are repeated explanations of Hb, Scr, ALB et al compared with Table 4. Similar problems in Table 6.

Answer: Thanks for your comment. As there are problems about repeated explanations of abbreviations in tables, we have summarized and added all the abbreviations used in this article as a short paragraph and added it before the declaration section in revised manuscript. The explanation of abbreviations in Tables was deleted.

G. Could you show us the exact numbers of response? CR #, VGPR# and so on.

Answer: Thanks for your comment. The exact number of patients achieved CR was 25, which was shown in Figure 2A. There were 44 patients with Very Good Partial Response and 11 patients with Partial Response.